Differences in resting state fMRI in rat under five different anesthetics

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Introduction

Resting state fMRI (rs-fMRI) has become a widely used and important tool in assessing functional networks of the brain. The spontaneous low frequency (< 0.1 Hz) fluctuations in blood oxygen level dependent (BOLD) signal have been proposed to reflect the intrinsic neuronal activity. [1] Therefore, rs-fMRI can provide valuable information of brain function in health and disease both in clinical [2] and preclinical [3,4] settings. However, translational value of preclinical results may be hindered by plausible effect of anesthesia to functional connectivity measurements. [5] The aim of this study was to investigate the possible differences in the resting state of rat brain under five different anesthetics.

Materials and methods

All animal procedures were approved by the National Animal Experiment Board of Finland. Altogether 52 adult male Wistar rats (350 ± 36 g) were used to study resting state with five different anesthetics (α-chloralose 60/30 mg/kg i.v., medetomidine 0.1 mg/kg/h i.v., thiobutabarbital 140 mg/kg i.p., urethane 1.25 g/kg i.p., and isoflurane 1.3 %). All rats were first anesthetized with isoflurane for femoral artery and vein cannulation. Tracheotomy was performed and pancuronium bromide (0.5 mg/kg/h i.v.) administered while attaching animal to ventilator. After surgery anesthesia was switched to either α-chloralose (n = 8), medetomidine (n = 7), thiobutabarbital (n = 12), urethane (n = 17), or isoflurane was continued (n = 8). MRI measurements were performed with 7 T Bruker PharmaScan. Functional data were acquired with single-shot SE-EPI (TR 2 s, TE 45 ms, 9 slices, 1.5 mm slice thickness, image matrix 64 x 64 and FOV 2.5 x 2.5 cm) where rs-fMRI scan consisted of 300 volumes (10 min). Temperature, heart rate and respiration were monitored. Blood samples were taken after the measurement and the arterial blood gas values were within normal physiological range (pCO_2 41.5 ± 6.1 mmHg, pO_2 145 ± 22 mmHg and pH 7.41 ± 0.06 including all animals). Data were motion and slice timing corrected, smoothed and co-registered using Statistical Parameter Mapping (SPM8) [6] and in-house made Matlab codes. Low-pass filtering was not performed and frequencies up to 0.25 Hz were studied. The Independent Component Analysis (ICA) was done with Group ICA of fMRI Toolbox (GIFT v2.0a). [7] Data dimensionality was reduced to 20 in subject level and to 30 in group level using Principal Component Analysis before doing ICA analysis. Statistical testing ($p < 0.05$) was performed with MANCOVAN Toolbox (v1.0), a part of GIFT.

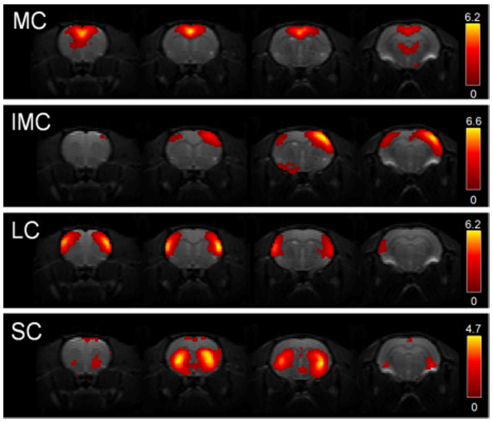

Figure 1. Four components in four slices shown from group ICA from all n = 52 rats with different anesthetics (thresholded with $z > 1$ and z-score bar shown on the right). The components are named as medial cortical (MC), intermediate cortical (IMC), lateral cortical (LC) and striatal (SC) component.

Results

The ICA including all n = 52 rats resulted in several interesting components, from which four components were chosen for further comparison (three cortical and one striatal component, see Figure 1). The initial MANCOVA stated significant differences between anesthetics in all cortical components, but not in striatal component. Additionally, a strong difference in functional network connectivity was detected. The results of univariate analysis ($p < 0.05$, FDR corrected) are summarized in Table 1. As can be seen from the table, the spatial and spectral differences of the independent components varied between the groups. In spectral data α-chloralose had increased power at 0.17 – 0.20 Hz, isoflurane at 0.04 – 0.07 Hz, medetomidine at 0.13 – 0.16 Hz and thiobutabarbital at 0.20 Hz or higher while comparing to each other. With urethane any clear and consistent spectral range with increased power against other groups was not seen. The amount of significantly different spatial voxels varied from few voxels to moderate cortical regions between groups. In univariate comparison the differences in functional network connectivity were limited to isoflurane group, which significantly differed from all other groups. No significant difference in striatal component was seen in any analysis.

Discussion

The results demonstrate that the resting state function of rat brain differs significantly in cortical networks between anesthetics. The spectral and spatial differences suggest that the spectral power and location of functional networks is modulated by the anesthetics in different way. The functional network connectivity between individual resting state networks was found to be significantly different with isoflurane anesthesia compared to any other anesthesia in this study. These findings support the earlier studies, where changes in physiology (e.g. diseases or drugs) have been suggested to reflect in functional networks. We conclude that the selection of anesthetic in animal resting state studies has to be carefully considered, especially when studying cortical networks.

Table 1. The statistically significant ($p < 0.05$, FDR corrected) differences between anesthetics related to spectral and spatial parameters of independent components (see Figure 1.) are summarized below. No significant differences in striatal component were detected.

	α-chloralose	Isoflurane	Medetomidine	Thiobutabarbital	Urethane
α-chloralose		LC, IMC	IMC	-	IMC
Isoflurane	MC, IMC		LC, IMC, MC	LC, IMC, MC	LC
Medetomidine	-	MC		IMC	MC, IMC
Thiobutabarbital	LC, IMC	LC, MC	LC, IMC		MC
Urethane	MC, IMC	MC, IMC	MC, IMC	LC, IMC, MC	

Spectral differences | Spatial differences

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